AD_______________

Award Number: W81XWH-07-1-0345

TITLE: Second-Generation Therapeutic DNA Lymphoma Vaccines

PRINCIPAL INVESTIGATOR: Larry W. Kwak, M.D., Ph.D.

CONTRACTING ORGANIZATION: University of Texas
M.D. Anderson Cancer Center
Houston, TX 77030

REPORT DATE: May 2008

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE
01-05-2008

2. REPORT TYPE
Annual

3. DATES COVERED
15 Apr 2007 -14 Apr 2008

4. TITLE AND SUBTITLE

Second-Generation Therapeutic DNA Lymphoma Vaccines

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-07-1-0345

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Larry W. Kwak, M.D., Ph.D.

E-Mail: lkwak@mdanderson.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Texas
M.D. Anderson Cancer Center
Houston, TX 77030

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The overall goal of our proposal is to develop a lymphoma vaccine for clinical study. Our vaccine strategy aims to activate immune cells that can recognize and eventually eliminate tumor cells. Lymphoma uniquely expresses a tumor-specific antigen termed "idiotype." By genetic modification, we linked this lymphoma antigen with a type of small proteins that are able to recruit immune cells. When given to animals, these vaccines profoundly induced tumor protection in lymphoma mouse models. In the proposed project, we will perform a series of animal experiments to choose the best vaccine formulation for a future clinical trial with lymphoma patients. We will also try to understand how the vaccine provokes the immune system to kill tumor cells. The selected vaccine should demonstrate the most potent antitumor effect that is mediated by immune cells. We will initiate the clinical trial, which is outisde the scope of this project, after these proposed studies are completed. Collectively, these studies will enable us to develop a more potent lymphoma vaccine and translate it into clinical application.

15. SUBJECT TERMS
Not Provided

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
					USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
U	U	U	UU	9	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

Annual Report

1. Introduction: Non-Hodgkin lymphomas (NHL) are a diverse group of lymphoproliferative neoplasms that differ in terms of their morphology, natural history, response to therapy, and prognosis. In the United States, NHL is the seventh most commonly diagnosed cancer and the sixth leading cause of cancer death. Although NHL is highly responsive to chemotherapy, the majority of patients relapse and eventually die of their disease. Therefore, novel, streamlined, potent therapeutic approaches to eliminate minimal residual disease are required to curb mortality from the disease. Therapeutic vaccination, in which a patient's immune system is "educated" to recognize and eliminate malignant cells, is a promising approach for eradication of minimal residual disease. The unique tumor immunoglobulin molecule from B-cell lymphoma, termed idiotype (Id), is an ideal tumor-specific antigen that can be used for vaccine generation. However, the development of an efficient vaccine formulation is largely limited by the inefficiency of vaccines in achieving potent and long-lasting antitumor immunities. Here, we developed a novel idioytpe vaccination strategy by (1) fusion of lymphoma idiotype antigen to ligands of chemokine receptors present on antigen-presenting cells (2) generation of a local inflammation at vaccination sites. The novel vaccination strategy allowed us to achieve potent and long-term antitumor effects in murine lymphoma models.

2. Results:

(i). Administration of cardiotoxin at vaccine injection sites significantly enhanced the therapeutic benefit of lymphoma idiotype DNA vaccine.

Study purpose: Evaluate the therapeutic advantage of cardiotoxin-combined idiotype DNA vaccination.

Experiment procedure: Groups of 10 mice were vaccinated with a lymphoma idiotype DNA vaccine by intramuscular injection of 50 μg plasmid DNA per mouse in the quadriceps. One group of mice was given 100 μL of 10 μM cardiotoxin 5 days before vaccination to induce a localized sterile inflammation at vaccine injection sites. Controls were injected with either PBS or cardiotoxin with an irrelevant vaccine. The prophylactic vaccination schedule includes a total of three rounds of immunization given at 2-week intervals. Two weeks after final vaccination, the mice were challenged with 2×10^5 A20 mouse lymphoma cells by intraperitoneal injection. In therapeutic studies, mice were first challenged with tumor cells, followed by vaccination on days 1, 3, 5, and 14. Tumor development and survival status were closely monitored. Data were analyzed by using the Kaplan-Meier method with the log-rank test to evaluate *P* values.

Results: Introduction of an inflammation at vaccine injection sites by cardiotoxin significantly improved the prophylactic antitumor effects of the lymphoma vaccine. This novel vaccination strategy resulted in 70% overall survival rate in tumor-challenged mice, whereas only 20% survival was achieved in those receiving vaccine alone. The local inflammation inducer cardiotoxin itself did not lead to significant tumor protection, as cardiotoxin alone or combining cardiotoxin with an irrelevant HIV vaccine failed to protect mice from tumor challenge (Figure 1). Likewise, the improved therapeutic antitumor effects against established tumor burden were also observed when cardiotoxin was given in advance at vaccine injection sites (Figure 2).

(ii). The novel vaccination approach activated potent memory antitumor effects

Study purpose: Determine the potential of the novel vaccination approach in activating long-term memory antitumor effects.

Experiment procedure: The disease-free mice that survived the tumor challenge at the end of prophylactic studies were further studied. Without giving any further treatment, we re-challenged

these mice with A20 lymphoma cells and monitored tumor development over the next 80 days to evaluate memory antitumor immunity.

Results: Figure 3 illustrates the data pooled from three individual studies, showing mice that received cardiotoxin–combined vaccination therapy significantly resisted tumor re-challenge. At the end of the re-challenge study, approximately 90% of mice were completely free of tumor. The mice given lymphoma vaccine only, however, developed tumors upon re-challenge, with a tumor-free rate of less than 40%. These findings suggest that introducing a local inflammation by cardiotoxin at vaccine injection sites facilitated the establishment of memory antitumor immunity, which in turn protected the mice from tumor re-challenge.

(iii). Introduction of an inflammation at vaccine injection sites is a technical innovation to enhance the therapeutic potency of lymphoma idiotype DNA vaccine.

Study purpose: Confirm the role of local inflammation inducers as an immune adjuvant in improving the antitumor potency of cancer vaccine.

Experiment procedure: We compared a group of adjuvant candidates head-to-head for their effects in improving the therapeutic potency of cancer vaccine. These candidates include local inflammation inducers cardiotoxin or crotoxin, as well as toll-like receptor agonists such as Poly IC (TLR3), MPL (TLR4), M001 (TLR7) and M003 (TLR7/8). Both cardiotoxin (6.8 μg/mouse) and crotoxin (10 μg/mouse) were given 5 days before vaccination to induce a local inflammation at vaccine injection sites; whereas all toll-like receptor agonists (50 μg/mouse) were given the next day of vaccination.

In other experiments, we examined the effectiveness of the novel vaccination strategy with three different lymphoma idiotype DNA vaccines including MIP3αsFv20, MCP3sFv20 and Defensin2βsFv20. In these studies, cardiotoxin was used to induce the inflammation at vaccine injection sites.

In all studies, the vaccination schedule includes three rounds of immunization, followed by intraperitoneal challenge with A20 mouse lymphoma cells as described before. Tumor development and survival were closely monitored, and data were statistically analyzed to evaluate the vaccine-induced antitumor effect.

Results: Introduction of a local inflammation at vaccine injection sites by either cardiotoxin or crotoxin significantly enhanced the lymphoma vaccine-induced tumor protection, achieving 70% to 90% disease-free survival in A20-challenged mice. However, at a literature-recommended dose (50 μg/mouse), none of the toll-like receptor agonists demonstrated the significance in boosting the efficiency of the lymphoma vaccine (Figure 4).

Figure 5 showed that using cardiotoxin to introduce an inflammatory reaction at vaccine injection sites resulted in long-term disease-free survival in at least 60% of all vaccinated mice no matter which lymphoma vaccine was given (upper panel, tumor-free rate; lower panel, overall survival rate), whereas the tumor-free survival rate was only 0% to 20% without cardiotoxin pretreatment. These results therefore support the general role of local inflammation inducers as an effective immune adjuvant for achieving the optimal therapeutic benefit of cancer vaccines.

3. Key Research Accomplishments:
(i) Comparing with toll-like receptor agonists, administration of inflammation inducers such as cardiotoxin or crotoxin at vaccination sites efficiently improved the therapeutic advantage of chemokine-fused lymphoma idiotype vaccines.

(ii) The novel vaccination strategy significantly enhanced both prophylactic and therapeutic antitumor effects of chemokine-fused lymphoma idiotype vaccines.

(iii) A long-term memory antitumor effect was notably activated by cardiotoxin-combined vaccination strategy.

(iv) Introduction of a sterile inflammation at vaccination sites could be developed into a general adjuvant strategy to boost the efficiency of cancer vaccines.

4. Conclusion

We developed a novel strategy to improve the therapeutic benefit of lymphoma idioytpe DNA vaccines by generating a sterile inflammation at vaccine injection sites. The local inflammation reaction was induced by intramuscular administation of cardiotoxin or crotoxin. Using a mouse lymphoma model, we observed this novel vaccination strategy consistently led to 60% to 90% disease-free long-term survival in tumor cell-challenged mice. Notably, 90% of the surviving animals were resistant to tumor re-challenge, highlighting the novel vaccination strategy facilitated the establishment of memory anti-tumor immunity. The adjuvant role of inflammation inducers was superior to that of toll-like receptor agonists in boosting idiotype vaccine-induced tumor protection in a mouse lymphoma model. In conclusion, generation of a local sterile inflammation at vaccine injection sites significantly improved the potency of idiotype vaccine in activating both instant and memory antitumor effects, which offers the novel vaccination strategy with clinical benefits of eliminating residual diseases, preventing relapse, improving disease-free survival, and curbing mortality in cancer patients.

5. Future plan for next year

Future studies will be focused on investigating mechanisms by which the novel vaccination strategy activates potent antitumor effects. In brief, we will evaluate both idiotype-specific cellular and humoral immunities activated by vaccination, and determine their roles in vaccine-induced tumor protection. We will also study the effect of the local inflammatory environment on the development of vaccine-induced anti-tumor immunities. These studies include examining the profile of cytokines and chemokines, as well as activation of antigen-presenting cells in the local inflammatory environment.

5. Reference

Zhu K, Qin H, Cha SC, Neelapu SS, Overwijk W, Lizee GA, Abbruzzese JL, Hwu P, Radvanyi L, Kwak LW, Chang DZ. Survivin DNA vaccine generated specific antitumor effects in pancreatic carcinoma and lymphoma mouse models. Vaccine. 2007 Nov 14;25(46):7955-61.

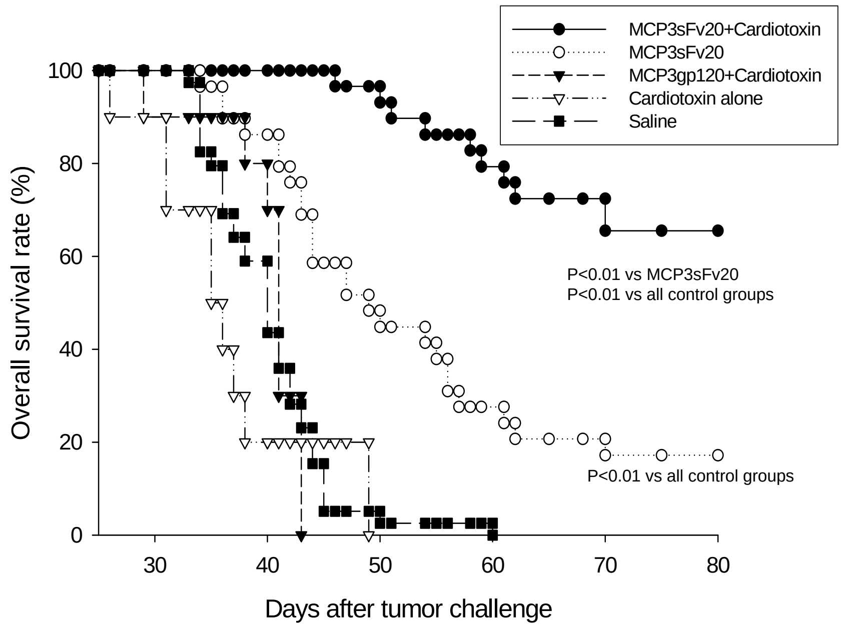

Figure 1: Administration of Cardiotoxin at vaccine injection sites significantly enhanced the therapeutic benefit of chemokine-fused lymphoma idiotype DNA vaccine.

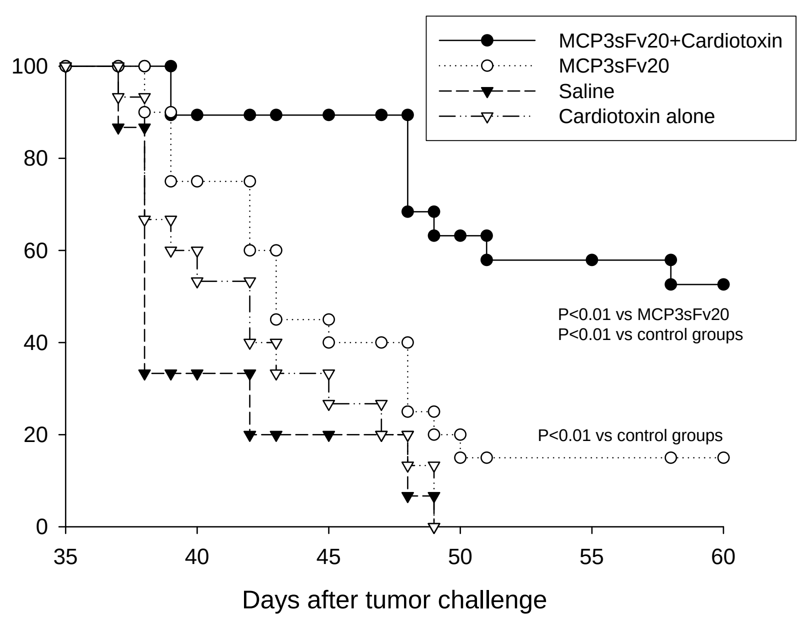

Figure 2: Combining cardiotoxin with chemokine-fused lymphoma idiotype vaccine significantly improved vaccine-induce tumor protection against established tumor burden.

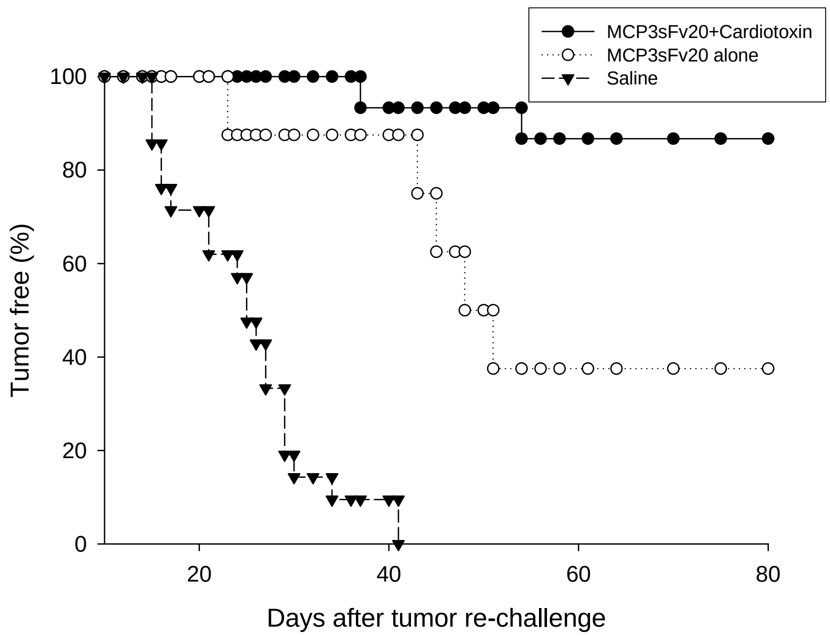

Figure 3: Cardiotoxin-combined vaccination strategy activated potent memory antitumor effects that protected mice from tumor re-challenge

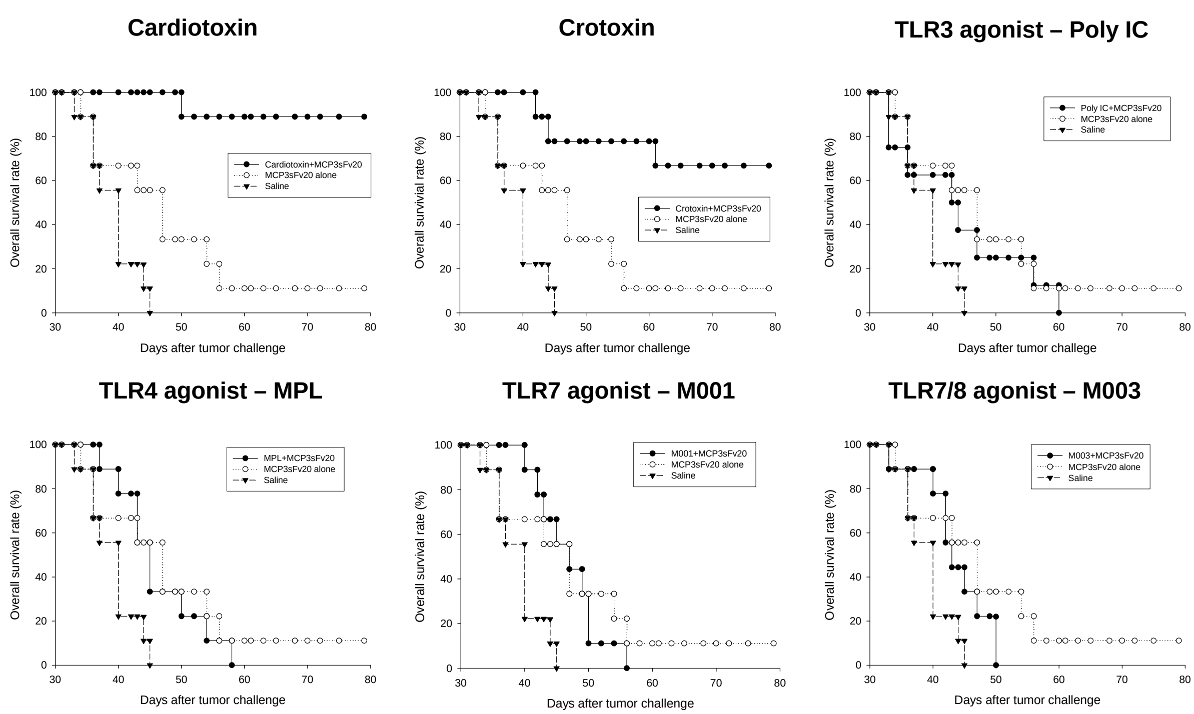

Figure 4: Local inflammation inducers Cardiotoxin and Crotoxin are potent adjuvants to enhance the antitumor effects of chemokine-fused lymphoma idiotype DNA vaccine

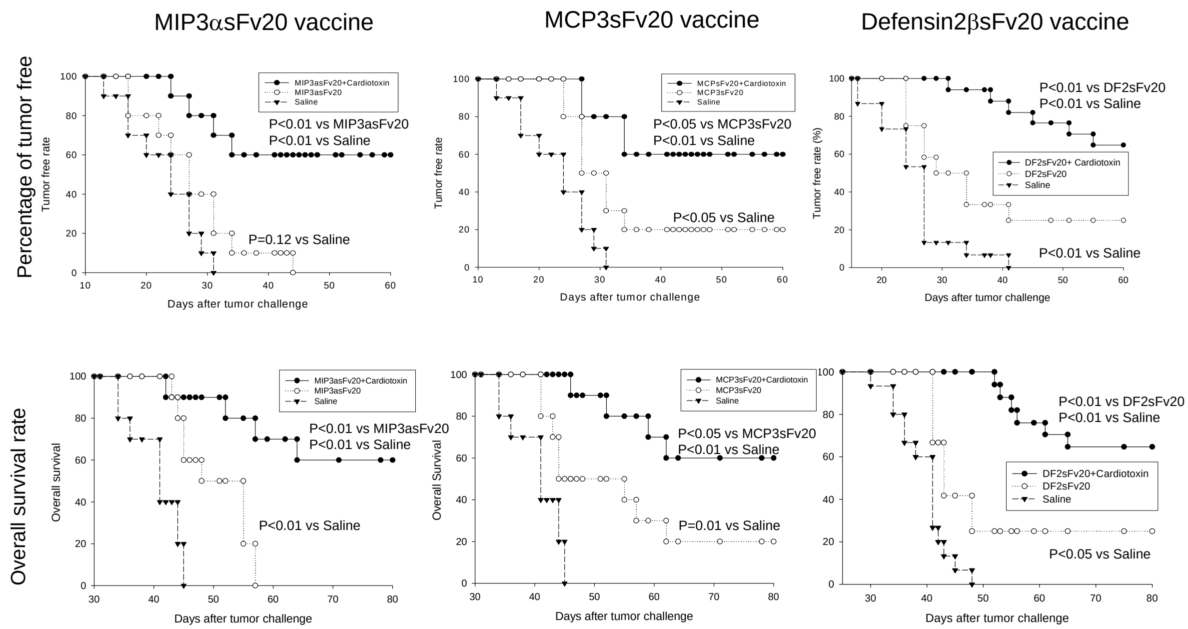

Figure 5: Introduction of a sterile inflmmation at vaccine injection sites is an efficient strategy to enhance the therapeutic potency of chemokine-fused lymphoma idiotype DNA vaccines